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Comparison of the effects of low-dose rosuvastatin on plasma levels of cholesterol and oxidized low-density lipoprotein in 3 ultracentrifugally separated low-density lipoprotein subfractions

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BACKGROUND: Plasma-oxidized (ox) low-density lipoprotein (LDL) is an atherogenic lipoprotein. The distribution of ox-LDL in plasma LDL subfractions and the effect of statins on this distribution have not been investigated in detail.

OBJECTIVE: We examined the distribution of cholesterol and ox-LDL in 3 ultracentrifugally separated plasma LDL subfractions and investigated the effects of a statin, rosuvastatin, on the levels of these lipoproteins.

MATERIALS AND METHODS: Thirty-one polygenic hypercholesterolemic subjects were included in this study. Levels of cholesterol and ox-LDL in 3 plasma LDL subfractions and plasma levels of remnant-like particle cholesterol, ox-LDL, and adiponectin were measured after 0, 3, 6, and 12 months of treatment with rosuvastatin. Sequential ultracentrifugation was performed to subfractionate plasma lipoproteins.

RESULTS: The mean daily dose of rosuvastatin over the 12 months of treatment was 2.9 ± 1.0 mg (mean ± standard deviation). The cholesterol subfraction distribution was 43 ± 10% as low-density LDL, 46 ± 8% as medium-density LDL, and 13 ± 5% as high-density LDL. Similarly, the distribution of ox-LDL was 31 ± 10% as low-density LDL, 48 ± 7% as medium-density LDL, and 22 ± 8% as

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high-density LDL. After 12 months of treatment with rosuvastatin, the level of cholesterol was significantly reduced in all 3 subfractions ($P < .0001$), as was the level of ox-LDL ($P < .0001$). Furthermore, the plasma cholesterol level in high-density lipoprotein$_2$ increased significantly.

CONCLUSIONS: The distribution of ox-LDL in plasma LDL subfractions was more skewed toward the denser subfractions, compared with cholesterol. Rosuvastatin treatment significantly reduced plasma levels of cholesterol and ox-LDL in all LDL subfractions.

Introduction

Oxidized (ox) low-density lipoprotein (LDL) plays a critical role in the initiation and progression of atherosclerosis,[1–3] functioning mainly in the arterial walls. Furthermore, the plasma level of ox-LDL has been reported to increase in patients with atherosclerotic disease.[4–9] Lipoproteins with densities ranging from 1.019 to 1.063 are designated as LDLs; therefore, density-based subfractions of LDLs can be identified.[10] Small, dense LDL particles are considered more atherogenic than large, less dense particles because their small size allows them to penetrate the intimal layer of arteries more easily.[11–15] Statins have been proven to be very useful for the primary and secondary prevention of coronary artery disease (CAD).[16–24] These drugs decrease not only plasma levels of LDL-cholesterol (LDL-C), but also those of ox-LDLs[25–29]; their effects on plasma cholesterol levels in LDL subfractions have also been reported.[30–33] In those studies, ox-LDL was reported to be preferentially found in dense LDL fractions[34] and to be reduced by rosuvastatin treatment.[35] However, in those studies, ox-LDL was measured using anion-exchange chromatography. Electronegative-LDL is rich in ox-LDL, but very heterogeneous.[36,37] A previous study measuring LDL reacting to an antibody against malonaldehyde (MDA)-modified LDL (MDA-LDL) showed that, unlike cholesterol, ox-LDLs preferentially distribute in the denser LDL fractions, and that simvastatin decreased the level of cholesterol, but not ox-LDL, in dense LDL fractions.[27] However, the effects of rosuvastatin on the distribution of LDL reacting to an antibody against MDA-LDL in LDL subfractions have not been described.

In this study, we investigated the distribution of cholesterol and ox-LDL in 3 ultracentrifugally separated plasma LDL subfractions and examined the effect of rosuvastatin on these fractions. We also examined the effect of rosuvastatin on the plasma levels of high-sensitivity (hs)-C-reactive protein (CRP), which is high in CAD patients,[38,39] and adiponectin (AN), an adipocyte cytokine that is low in CAD patients[40–42]; the intima-media thicknesses (IMTs) of the common carotid arteries were also investigated.

Materials and methods

This study was approved by the ethics committee of Tokai (Japan) University Hospital. The trial was started after obtaining written informed consent from all participants.

Subjects and study protocol

The subjects were outpatients having polygenic hypercholesterolemia and attending the Department of Cardiology, Tokai University Hospital. Polygenic hypercholesterolemia was defined as hypercholesterolemia, exclusive of familial hypercholesterolemia, familial combined hyperlipoproteinemia, and secondary hypercholesterolemia; the patients did not have any blood relatives with hypercholesterolemia. The LDL-C and triglyceride plasma levels of the patients were >160 mg/dL and <200 mg/dL, respectively. Patients who took drugs affecting plasma lipid levels, such as β-blockers and diuretics, and those taking antioxidants, such as vitamin E, were excluded from the study. The trial was started after 2 months of dietary treatment involving a daily energy intake of 1800 kcal, daily fat intake equivalent to 25% of the total energy intake, a polyunsaturated/saturated fat ratio of 1.0, and a daily cholesterol intake of 300 to 400 mg. The patients received an initial rosuvastatin dose of 2.5 mg/day for 1 month; in the next month, the dose was increased to 5 mg/day, and in the third month, to 10 mg/day to reduce plasma LDL-C levels to <130 mg/dL. After 3 months of treatment, the rosuvastatin dose was fixed for each patient until the end of the trial, depending on how each reacted to the drug. The patients visited our outpatient clinic every month for side-effect assessments; laboratory tests were conducted every 3 months. Ultrasonic examinations of the carotid arteries were performed at the start and end of the trial.

Laboratory procedures

Patient blood, after 12 h of fasting, was collected into ethylenediaminetetraacetic acid–containing tubes and immediately separated by centrifugation at 4°C. Samples for measuring MDA-LDL were stored at −80°C until measurement. Plasma lipid concentrations were enzymatically measured using an autoanalyzer. Plasma LDL-C levels were measured directly, rather than calculated.[43] The levels of apo(-lipo)proteins were estimated using a turbidimetric immunoassay.[44] The amount of cholesterol in remnant-like particle cholesterol (RLP-C), similar to triglyceride (TG)-rich lipoprotein remnants, was measured using an immunoprecipitation method with monoclonal antibodies against apo(protein) AI and B-100.[45] LDL reacting to an antibody